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Tree biomass allometry during the early growth of Norway spruce (*Picea abies* (L.) Karst) varies between pure stands and mixtures with European beech (*Fagus sylvatica* L.)

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Abstract

In this paper we report an investigation of how forest stand mixture may affect biomass allometric relationships in Norway spruce (*Picea abies* (L.) Karst). Analysis of aboveground biomass data was conducted for 50 trees. Twenty-five sample trees were from a pure Norway spruce stand and the remainder were taken from a mixed stand of Norway spruce with European beech (*Fagus sylvatica* L.). ANCOVA results demonstrated that individual tree biomass allometry of the pure stand significantly differed from that of the mixed stand. Allometric characteristics depended on the biomass component recorded and the type of biomass predictor used. When predicted by DBH and/or height, the total aboveground biomass of mixed stand trees was significantly less than that for pure stand ones. This 'apparent' lower aboveground biomass was attributed to the lower branch and needle biomass proportions of trees growing in mixed stands. The findings indicate that caution should be exercised when applying biomass allometric models developed from pure stands to predict tree biomass in mixed stands (and vice versa), as such data treatment may introduce significant bias.

Key words: species composition effect, biomass partitioning, mixture, aboveground biomass, allometric equation

Introduction

Tree biomass allometry represents one of the most important tools available for estimating carbon sequestration in forest biomass, and is widely used when appropriate forest inventory data is available (Brown 2002, Picard et al. 2012). Allometric models are based on regression relationships that use tree dimensions (e.g. diameter at breast height, tree height, root collar diameter) to predict biomass (Baskerville 1972, Parresol 1999). The variation in such regression models is believed to be attributable to genetic factors (Enquist et al. 1999), abiotic environmental conditions (Delucia et al. 2000) and biotic influences such as competition for resources in the immediate environment (Copenhaver and Tinker 2014).

It is well established that biomass allometric models are site and species specific. Thus at a species level (Enquist et al. 1998, Pretzsch 2006), the genotype dictates how trees respond to competition and environment, thereby influencing the allometric characteristics of the phenotype. Wood density, which is genetically controlled (Rozenberg et al. 2001) has been demonstrated to affect allometric characteristics (Enquist et al. 1998) and has also been successfully used to improve the accuracy of prediction, especially in multispecies allometric models (Chave et al. 2014). It appears that because of genetic similarity, allometric characteristics of some species are not greatly influenced by the method of forest regeneration. This is demonstrated by Kauppi et al. (1988) in their study of coppiced and planted downy birch (*Betula pubescens* Ehrh.), whose findings are consistent with research conducted on other species (for example see: Harrington and Fownes (1993), António et al. (2007)). The site specificity of allometric models is attributable to the fact that each forest site has unique environmental conditions (e.g. soil, precipitation, temperature) and each tree species has unique competitive characteristics. Soil

properties have been shown to influence biomass allometry (de Castilho et al. 2006, Dutcă et al. 2014), as has climate (Callaway et al. 1994, Lines et al. 2012). However, the variability of biomass-DBH relationships is most commonly influenced by heterogeneity in environmental conditions. Such variability may destabilise height-DBH relationships and render them unsafe for making biomass predictions. Chave et al. (2014) found that the effects of climatic conditions and tree species were apparently 'removed' when biomass was modelled using a combination of DBH, height and wood density predictors. This is consistent with a view that much of the environmental effect is explainable by height-DBH relationships, whereas species effects are statistically related to wood density variation. In a study of Amazonian species, Baker et al. (2004) similarly found that much of the variance in wood density could be attributed to species and genus effects.

A review of the literature highlights considerable variation and conflicting findings from studies that evaluate the effect of stand density on tree biomass allometry. Usually stronger competition accelerates growth (which may affect wood density) and changes the biomass allocation among tree organs (Poorter et al. 2012). However, some studies report that stand density has no biomass allometric effect (António et al. 2007), whereas workers studying a variety of broadleaved and coniferous species report the significant influence of stand density and, therefore, stand competition on tree allometry (see for example: Enquist et al. (1998), Copenhaver and Tinker (2014)).

In their study of two oak species, Saha and co-workers (2014) demonstrated that interspecific competition varied in its influence on growth and stem quality when compared to intraspecific competition. Conversely, (Pretzsch 2014) did not find any significant differences in height-DBH allometry, although European beech trees

proved to be more effective in gap filling, and therefore imposed greater lateral competition pressure when compared to Norway spruce trees (Dieler and Pretzsch 2013). Norway spruce (*Picea abies*, L. Karst) is one of the most common tree species in Europe, and frequently occurs in mixtures with European beech (*Fagus sylvatica* L.). Mixing Norway spruce with European beech has been shown to stimulate growth in both species and greater biomass production than otherwise occurs in pure stands (Pretzsch and Schütze 2009). This phenomenon can therefore be used to enhance carbon sequestration in forests. As a result of the different morphological plasticity of the two species, crown allometry has been demonstrated to be significantly different in mixed stands when compared to pure stands. This difference has also been shown to be species 'specific' (Pretzsch 2014). Despite extensive research to explore differences between pure and mixed stands, it is not yet understood how stand mixtures influence tree biomass allometric models. Understanding how biomass allometry is affected by species composition is scientifically important and has practical application for silviculture and for carbon sequestration to mitigate climate change. Because developing allometric models is labour intensive, tree sampling is often only conducted in pure stands for reasons of convenience. However, these models are applied in forestry practice regardless of whether the trees are growing in pure or mixed stands and without awareness of the likelihood that such models will introduce bias.

This study therefore aimed to investigate how tree development in pure or mixed stands (with European beech) affected the biomass allometry of Norway spruce. We hypothesized that: (i) tree biomass allometry of Norway spruce may be influenced by interspecific competition which, in turn, may result in differences between allometric relationships for pure Norway Spruce and for mixtures with

European beech; (ii) the aboveground biomass of Norway spruce grown in mixed stands would be less than that for trees of equivalent diameter and/or height grown in pure stands due to greater lateral competition from European beech; and (iii) using both DBH and height to predict biomass would not be sufficient to compensate or offset for species composition effects.

Materials and Methods

Location of the study

Measurements were taken in two neighbouring forest stands, one pure and one mixed, located in Brasov County, Romania (Table 1). The climate is cold and temperate (Dfb according to the updated world climate classification of Köppen and Geiger (Kottek et al. 2006)). The average temperature is 5.3°C and annual mean precipitation is 850 mm in both forest stands. The two stands were selected as being appropriate candidates for the study, showing similar DBH range and environmental conditions. The mixed stand consisted of naturally regenerated European beech and planted Norway spruce. Beating up was undertaken in both stands so that gaps resulting from losses to seedling mortality were filled with successive generations of Norway spruce seedlings. The pure stand was established as a plantation solely of Norway spruce with subsequent generations of gap-fill seedlings. However, natural regeneration of European beech occurred on approximately 10% of the stand area.

In each stand, a sample plot of 400 m^2 was established, and the DBHs and heights of all trees were recorded. In the mixed stand, mean DBH for European beech was found to be 7.5 cm compared to 8.3 cm for Norway spruce. Mean height of European beech, at 8.2 m, was also less than that for Norway spruce (8.5 m), but the difference was not so marked as for DBH.

Measurements

Aboveground biomass was measured for a total of 50 trees in July 2015 (15 trees from each stand) and July 2016 (10 trees from each stand). Twenty-five Norway spruce trees were sampled in each of the pure and mixed stands. The trees were selected based on the following criteria: (i) in the mixed stand, only Norway spruce trees adjacent to European beech trees were included for study; (ii) supressed trees, or those showing signs of affected structural integrity or disease were avoided in both stands; (iii) only Norway spruce trees of relatively similar height to the neighbouring trees were included for study; (iv) a lognormal DBH distribution was applied for tree selection.

After felling, the stem and branch (including needles) components of each tree were separated and the fresh biomass of each component was measured in the field using an electronic scale (0.1 g precision). Wood samples from the stem were taken and dried at 80°C (to constant weight), to determine dried biomass. One or two branches were also randomly selected from each whorl and pre-dried in the laboratory, so that needles could be more easily separated from branches. Needles and branches were then further dried at 80°C (to constant weight). The dried biomass proportion of each component (stem, branches and needles) was then calculated.

DBH was measured *in situ*, at 1.3 m from the ground, with a forest calliper (1mm accuracy), and recorded as the mean of two perpendicular diameter measures. Height was recorded after felling using a meter tape and measurements were made to a similar accuracy of ±1mm.

The DBH of the 50 sampled trees ranged between 1.3 and 13.0 cm (trees sampled from mixed stand: 2.3 to 13.0 cm; trees sampled from pure stand: 1.3 to 12.9 cm) and height ranged from 2.1 to 13.1 m (trees sampled from mixed stand: 2.9 to

13.1 m; trees sampled from pure stand: 2.1 to 12.9 m). The total aboveground biomass of mixed stand trees ranged from 1309 to 40266 g and from 1768 to 49722 g for pure stand trees.

Norway spruce trees in pure stands are hereafter referred to as “pure trees” and those growing adjacent to European beech, as “mixed trees”. For each tree, the stem, branch and needle biomass were measured in the field. The dried biomass categories were: stem biomass (ST), branch biomass (BR), needle biomass (ND) and aboveground biomass (AB – calculated as a sum of ST, BR and ND).

Competition index

Tree competition index was calculated for each sampled tree, based on DBH of sampled tree (d), DBH of i-th competitor tree (d_i), number of competing trees within a radius of 3.0 m (n) and the distance between sampled and i-th competitor tree ($dist_i$) using the formula proposed by Hegyi (1974):

$$CI = \sum_{i=1}^{n} \frac{d_i}{d \times dist_i} \quad (1)$$

Different neighbouring species exhibit varying levels of crowding and shading (Canham et al. 2004). However, a species effect was not included in the competition index to avoid concealing the effect of the different neighbouring species on biomass allometry and, in turn, reducing the possibility of type II error.

Statistical analysis

All variables were subject to natural log-transformation prior to analysis and model development. The following annotations are used throughout the paper:

– ln(AB), natural logarithm of aboveground biomass (AB);

– ln(ST), natural logarithm of stem biomass (ST);

– ln(BR), natural logarithm of branch biomass (BR);

– ln(ND), natural logarithm of needle biomass (ND);

– ln(DBH), natural logarithm of diameter at breast height (DBH);

– ln(H), natural logarithm of height (H);

– ln(DBH^2H), natural logarithm of squared DBH multiplied with H.

Using ln(DBH) and ln(H), together within the same model, as distinct independent variables revealed that they are highly collinear, yielding a variance inflation factor greater than 10 (VIF = 10.52). However, ANCOVA is sensitive to collinearity of covariates (Tabachnick and Fidell 2013). As a consequence, the two covariates were merged into a single term, ln(DBH^2H). Moreover, two covariates, ln(DBH) and ln(DBH^2H), were found to be non-linear. This was corrected by squaring the covariates.

Analysis of covariance (ANCOVA)

ANCOVA was used to determine the main effect of tree species composition on biomass allometry. The main effect takes into account intercept differences while being conditioned on similar slopes (therefore correcting for any interaction). The ANCOVA model was:

$$Y = b_0 + b_1 T_d + b_2 SC + \varepsilon \quad (2)$$

Here the dependent variables (Y) are expressions for biomass ln(AB), ln(ST), ln(BR) and ln(ND), the covariates (T_d) are the tree dimension variables $\ln(DBH)^2$, ln(H) or $\ln(DBH^2H)^2$. Species composition (SC) was the categorical independent variable (for which an effect was tested) and was one of two values, either ‘pure’, or, ‘mixed’. The errors $\varepsilon \sim N(0, \sigma^2)$ are assumed to be normally distributed and independent. Furthermore, because tree competition may have a significant fixed effect on biomass we included the tree competition index (CI) as an additional covariate:

$$Y = b_0 + b_1 T_d + b_2 CI + b_3 SC + \varepsilon \quad (3)$$

The assumptions of ANCOVA (i.e. normality, homogeneity of variance, independence, linearity and homogeneity of slope) were checked. Homogeneity of the slopes was tested by adding an interaction term (see 'interaction effect'). The interactions are presented along with ANCOVA results.

When included more than one covariate, type II sum of squares ANCOVA was used to test the main effects because this provides a more powerful test and avoids type I errors (i.e. reduces the probability of a false rejection of the null hypothesis in F-tests).

Interaction effect

The interaction effect is sensitive to regression slope differences and, when comparing two allometric equations, importantly highlights differences in relative growth rates between the two groups (i.e. the differences in allometric scaling). The following models were used to test the interactions for Eq. 2:

$$Y = b_0 + b_1 T_d + b_2 SC + b_3 (T_d \times SC) + \varepsilon \quad (4)$$

And for Eq. 3:

$$Y = b_0 + b_1 T_d + b_2 CI + b_3 SC + b_4 (T_d \times CI) + b_5 (T_d \times SC) + b_6 (CI \times SC) + b_7 (T_d \times CI \times SC) + \varepsilon \quad (5)$$

The annotations of variables are similar to Eq. 2 and 3.

Prediction bias

Bias was calculated as the difference between predicted biomass of pure trees ($\hat{B}_p$) and the predicted biomass of mixed trees ($\hat{B}_m$) as produced from power function allometric models (back transformed equations), for any given value of independent variable (covariate):

$$Bias\ (\%) = \frac{\hat{B}_p - \hat{B}_m}{\hat{B}_p} \times 100 \quad (6)$$

Back transformation, from linear to 'power' form equation, produces a bias (Baskerville 1972). A correction factor (*CF*) based on residual standard error (*RSE*) was used to correct for bias (Sprugel 1983):

$$CF = \exp(\frac{RSE^2}{2}) \quad (7)$$

The bias resulting from the main effect was calculated for models with significant ANCOVA and non-significant interaction. Non-significant interactions indicated that slopes were not significantly different, and therefore the differences between pure and mixed trees were mainly attributable to intercept differences. Because the slopes were not completely identical, a random intercept mixed effect model was applied (imposing identical slopes for both pure and mixed trees but allow intercept to vary). The result of imposing identical slopes is that bias remained constant. Thus, this was represented as percentage bias.

Statistical analyses were conducted in R (R Development Core Team 2016) with the RStudio (RStudio Team 2016) interface and using 'car' (Fox and Weisberg 2011) and 'nlme' (Pinheiro et al. 2017) packages.

Results

Differences between biomass allometry of pure and mixed trees

The differences between pure and mixed trees were assessed using tree dimension covariates only (i.e. $\ln(DBH)^2$, $\ln(H)$ and $\ln(DBH^2H)^2$). ANCOVA assumptions were satisfied by all models, except for ln(ST) predicted by $\ln(DBH^2H)^2$, which showed significant interaction. Neither $\ln(DBH)^2$, $\ln(H)$ nor $\ln(DBH^2H)^2$ as covariates demonstrated any other significant interaction with respect to regression lines for pure and mixed trees (Fig. 1 and Table S1). Using $\ln(DBH)^2$ as the covariate, ANCOVA results indicated that intercept values of pure and mixed trees were significantly

different for all biomass categories, except stem biomass. Only stem biomass predicted by $\ln(DBH)^2$ demonstrated nearly similar patterns for both pure and mixed trees. Modelling biomass on $\ln(DBH)^2$, $\ln(H)$ or $\ln(DBH^2H)^2$ revealed that in mixed stands (for the same value of covariate) trees exhibited lower aboveground biomass. Significant differences for $\ln(AB)$ predicted by $\ln(DBH)^2$, $\ln(H)$ and $\ln(DBH^2H)^2$ between pure and mixed trees, was found to be due to significant differences in branch and needle biomass.

The relationship between independent variables

To understand the biomass allometric differences between pure and mixed trees presented earlier, it is important to examine the relationship between independent variables. The relationships between $\ln(H)$ and $\ln(DBH)^2$ were similar for both stands (Fig. 2).

Biomass component proportions of total aboveground biomass for pure and mixed trees

The proportion of components (stem, branches and needles) out of total aboveground biomass (Fig. 3) was significantly different in pure and mixed stands. Stem biomass proportion was significantly greater for trees growing in the mixed stand compared to the pure stand. However, branch biomass proportion was significantly less for mixed trees. The proportion of needle biomass was also less, although not significant so (Pearson $p = 0.077$).

Including the tree competition index as covariate

The tree competition index (CI) was introduced as a covariate to further reduce the type II error (false negative) in ANCOVA. This however did not markedly change overall results. As before, ANCOVA demonstrated significant differences between intercepts for pure and mixed trees (AB=*f*(DBH, CI); AB=*f*(H, CI); AB=*f*(DBH, H,

CI); BR=*f*(DBH, CI); BR=*f*(H, CI); BR=*f*(DBH, H, CI), ND=*f*(DBH, CI) and ND=*f*(DBH, H, CI)). However, the main SC effect was weaker after inclusion of CI as covariate in ANCOVA (Table 2).

Competition index (CI) was not found to be significantly correlated with either DBH (Pearson $p = 0.153$) nor H (Pearson $p = 0.324$). Also, there was no significant CI difference between pure and mixed stands (ANOVA $p = 0.456$).

Prediction bias

The significant differences between allometry of pure and mixed trees was a source of bias (systematic error caused by the type of species composition) in biomass prediction, with an effect that ranged from -15.47 to -29.91% (Table 3). The scale of bias demonstrates the extent to which predicted biomass of mixed trees differed to that for pure stand ones. For any given DBH, the AB biomass of trees growing in mixed stand was 15.47% less compared to that in pure stand. Furthermore, for any given DBH, the BR and ND biomass of mixed trees was 28.11 and 19.93% respectively less than that for pure stand trees (Table 3). Bias was highest when both DBH and H were jointly used to predict biomass (i.e. by using $\ln(DBH^2H)^2$ covariate).

Discussion

Results demonstrated significant differences in the biomass allometry for pure and mixed trees for some widely used biomass categories and predictors. These differences were further reflected in biomass prediction biases.

Why was the biomass allometry of pure trees different from that of mixed trees?

The differences in the AB allometry of pure and mixed trees were attributable to differences in each of the AB components (ST, BR and ND) and were also correlated

with the independent ‘predictor’ variables (DBH and H). Stem biomass allometry was found to be similar for pure and mixed trees when DBH, H or DBH and H was used as predictors (Fig. 1). However, the ST proportion of AB biomass was significantly greater in mixed stands, due to less overall AB compared to that of pure stands. These results are consistent with Poorter et al. (2012) who observed for ‘crowded’ stands that stem biomass forms a significantly greater proportion of overall tree biomass and, conversely, that the proportion of leaf and root biomass was significantly less.

The relationship between H and DBH may affect stem biomass allometry, so for the same DBH value, greater H can yield more stem biomass. Results, however, demonstrated a similar pattern of H-DBH relationship for both pure and mixed trees which. These H-DBH relationship findings are consistent with other publications (see Pretzsch (2014), Drössler et al. (2015)). However, Loewe et al. (2013) demonstrated significantly different patterns of stem biomass allometry between pure and mixed stands of cherry trees (*Prunus avium* L.), but only when grown in mixture with black locust (*Robinia pseudoacacia* L.). Thus, it appears that differences in stem biomass allometry may be influenced by mixture type. For example, *Robinia* sp. could influence neighbouring tree species through nitrogen fixation, as demonstrated by Forrester et al. (2006) for *Eucalyptus* mixtures.

In mixed stand trees the biomass proportion represented by branches was reduced and, consequently, the proportion represented by leaf biomass was also reduced. This could be attributed to greater lateral competition pressure imposed by European beech (Pretzsch et al. 2010, Pretzsch 2014). These results are consistent with Dieler and Pretzsch (2013), who reported for larger and older trees that the crowns of Norway spruce in mixture with European beech were smaller when compared to pure stands. However, such responses to mixture were species related

since in the same study, European beech were found to have larger crowns when grown in mixture. Moreover, Forrester et al. (2017a) found that 13 out of 17 tree species had smaller crowns under stronger tree competition and the crown response to competition was related to species wood density. Under similar competition, the crown reduction was stronger in those species exhibiting lower wood density. Therefore, differences in biomass allometry between pure and mixed stands may depend on the morphological plasticity of species which, in turn, infers that levels of biomass prediction bias due to SC (species composition) may also be determined by species and mixture type. Therefore, despite having similar ST, AB was significantly lower in the mixed stand due to lower proportions of BR and ND biomass.

Although in this tree-level study the AB biomass was found to be significantly less in mixed stands, Pretzsch and Schütze (2009) found that the opposite relationship held for their stand-level study of a similar mixture type. Thus, despite each tree having less AB biomass for the same DBH or H, mixed stands successfully produce more biomass per unit area than pure stands. This was shown to be related to the better capacity of mixed stands to exploit canopy space (Pretzsch 2014, Jucker et al. 2015), allowing more trees to grow per unit area (Pretzsch and Biber 2016), and to the greater mean tree volume of Norway spruce in mixed stands (Pretzsch and Schütze 2014).

From a perspective of biomass allocation, our results suggest that the Norway spruce trees experienced competition from European beech when growing in mixture. Furthermore, the smaller crowns of mature Norway spruce trees in the same type of mixture (Dieler and Pretzsch 2013, Forrester et al. 2017a) suggests that competition may remain the main type of interaction during the lifetime of the stand.

Within this study, no significant CI difference between pure and mixed stand was observed, although the species effect was not included (Eq. 1). Incorporating the species effect, such as the light transmission coefficient (0.8 for European beech and 1.0 for Norway spruce, (Pretzsch and Schütze 2009)) in Eq. 1, the difference became significant (ANOVA $p = 0.033$). It may be that Norway spruce trees encountered greater competition in mixed stand, due to stronger European beech effects (as observed by Dieler and Pretzsch (2013)). Also, unlike in older secondary forests (Wang et al. 2011), in our study competition index was not significantly related to either DBH or H. This was because in our study the competing trees were of relatively of similar size to the core tree (sampled tree).

Concerning predictive capabilities of independent variables in pure and mixed stands

DBH was least affected by prediction bias in mixed stands, and when compared in allometric models developed for pure stands (Table 3). Tree height is potentially attractive as a biomass indicator due to its ease of measurement in aerial images (Jucker et al. 2017). However, when height alone was used, the AB biomass prediction bias between pure and mixed stands (at approximately 19%, Table 3) was greater than using DBH alone (approximately 15%, Table 3). Biomass predictions that include height and wood density as covariates with DBH can be effective in offsetting site and species effects (Wirth et al. 2004, Chave et al. 2014, Forrester et al. 2017b). This is related to H-DBH relationship being one of the main influences of biomass-DBH site variability and to species variations in wood density. Although the H-DBH relationship has been shown to be sensitive to environmental effects (Feldpausch et al. 2012), results reported here demonstrate an invariant H-DBH

allometry in pure and mixed stands that is similar to findings reported by Pretzsch (2014).

However, assuming that species composition (SC) effect in the ln(AB)-ln(DBH) relationship is due to H-DBH variation, it was anticipated that including height (using the $\ln(DBH^2H)^2$ covariate) would offset the SC effect compared to using DBH alone (i.e. $\ln(DBH)^2$). Our results suggest the opposite effect. When height was included the SC effect became more significant (SC effect on aboveground biomass, based on $\ln(DBH)^2$: $p = 0.0019$; SC effect with covariate $\ln(DBH^2H)^2$: $p = 0.0008$, see Fig. 1), thereby, accentuating the difference between pure and mixed trees. Although intriguing, this occurred because by including height, the proportion of explained within-group variance was increased, and therefore the remaining SC effect became clearer. Thus, by including height, the within-group variance was reduced, in favour of between-group variance (reducing the type II error in ANCOVA). This was most evident for stem biomass, where SC effect increased to a near significant level (SC effect on stem biomass, based on $\ln(DBH)^2$: $p = 0.602$; SC effect with covariate $\ln(DBH^2H)^2$: $p = 0.051$, see Fig. 1). Overall, this explains why the bias caused by SC effect (Table 3) was greater when both DBH and H were used to predict biomass. Therefore, the findings of this study demonstrate that SC can influence biomass-DBH relationships, and this variation may not be offset by including height in allometric models.

Nevertheless, the SC effect decreased when the competition index (CI) was included as a covariate (e.g. SC effect on aboveground biomass based on $\ln(DBH)^2$: $p = 0.002$; SC effect with covariates $\ln(DBH)^2$ and CI: $p = 0.005$, see Fig. 1 and Table 2). This was also observed for the other models. Therefore, unlike height, the CI explained part of SC effect. However, the SC effect remained significant regardless of

whether CI was included, thus indicating that CI explained only a small proportion of the variation attributable to species composition.

The implications of research findings for forestry practice

Biomass allometric models are often developed using data from one type of species association (i.e. from pure stands or mixed stands). However, in forestry practice these models are applied regardless of whether trees grow in pure or mixed stands. Results for this study demonstrate that allometric models predicting aboveground biomass were significantly different in pure and mixed stands. This difference resulted in a constant bias in biomass prediction that was due to species composition and managers should be aware of this potential bias.

Conclusions

The findings of this study demonstrate that biomass allometry may be significantly influenced by species association/mixture effects. When grown in mixture with European beech, Norway spruce aboveground, branch and needle biomass was significantly reduced when predicted by DBH, H or both, in comparison to Norway spruce in pure stands. These differences were a source of significant prediction bias. It is therefore recommended that the use of pure monospecific biomass models are not transposed to mixtures (or vice versa), unless they are first validated and/or an appropriate correction factor is determined.

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References

António, N., Tomé, M., Tomé, J., Soares, P., and Fontes, L. 2007. Effect of tree, stand, and site variables on the allometry of Eucalyptus globulus tree biomass. Can. J. For. Res. **37**(5): 895–906. doi:10.1139/X06-276.

Baker, T.R., Phillips, O.L., Malhi, Y., Almeida, S., Arroyo, L., Di Fiore, A., Erwin, T., Killeen, T.J., Laurance, S.G., Laurance, W.F., Lewis, S.L., Lloyd, J., Monteagudo, A., Neill, D.A., Patino, S., Pitman, N.C.A., M. Silva, J.N., and Vasquez Martinez, R. 2004. Variation in wood density determines spatial patterns inAmazonian forest biomass. Glob. Chang. Biol. **10**(5): 545–562. Blackwell Science Ltd. doi:10.1111/j.1365-2486.2004.00751.x.

Baskerville, G.L. 1972. Use of Logarithmic Regression in the Estimation of Plant Biomass. Can. J. For. Res. **2**(1): 49–53. NRC Research Press Ottawa, Canada . doi:10.1139/x72-009.

Brown, S. 2002. Measuring carbon in forests: current status and future challenges. Environ. Pollut. **116**(3): 363–372. doi:10.1016/S0269-7491(01)00212-3.

Callaway, R.M., DeLucia, E.H., and Schlesinger, W.H. 1994. Biomass Allocation of Montane and Desert Ponderosa Pine: An Analog for Response to Climate Change. Ecology **75**(5): 1474–1481. Ecological Society of America. doi:10.2307/1937470.

Canham, C.D., LePage, P.T., and Coates, K.D. 2004. A neighborhood analysis of canopy tree competition: effects of shading versus crowding. Can. J. For. Res. **34**(4): 778–787. NRC Research Press Ottawa, Canada . doi:10.1139/x03-232.

de Castilho, C. V., Magnusson, W.E., de Araújo, R.N.O., Luizão, R.C.C., Luizão, F.J., Lima, A.P., and Higuchi, N. 2006. Variation in aboveground tree live biomass in a central Amazonian Forest: Effects of soil and topography. For. Ecol. Manage. **234**(1–3): 85–96. doi:10.1016/j.foreco.2006.06.024.

Chave, J., Réjou-Méchain, M., Búrquez, A., Chidumayo, E., Colgan, M.S., Delitti, W.B.C., Duque, A., Eid, T., Fearnside, P.M., Goodman, R.C., Henry, M., Martínez-Yrízar, A., Mugasha, W.A., Muller-Landau, H.C., Mencuccini, M., Nelson, B.W., Ngomanda, A., Nogueira, E.M., Ortiz-Malavassi, E., Pélissier, R., Ploton, P., Ryan, C.M., Saldarriaga, J.G., and Vieilledent, G. 2014. Improved allometric models to estimate the aboveground biomass of tropical trees. Glob. Chang. Biol. **20**(10): 3177–3190. doi:10.1111/gcb.12629.

Copenhaver, P.E., and Tinker, D.B. 2014. Stand density and age affect tree-level structural and functional characteristics of young, postfire lodgepole pine in Yellowstone National Park. For. Ecol. Manage. **320**: 138–148. doi:10.1016/j.foreco.2014.03.024.

Delucia, E.H., Maherali, H., and Carey, E. V. 2000. Climate-driven changes in biomass allocation in pines. Glob. Chang. Biol. **6**(5): 587–593. Blackwell Science Ltd. doi:10.1046/j.1365-2486.2000.00338.x.

Dieler, J., and Pretzsch, H. 2013. Morphological plasticity of European beech (Fagus sylvatica L.) in pure and mixed-species stands. For. Ecol. Manage. **295**: 97–108. doi:10.1016/j.foreco.2012.12.049.

Drössler, L., Övergaard, R., Ekö, P.M., Gemmel, P., and Böhlenius, H. 2015. Early development of pure and mixed tree species plantations in Snogeholm, southern Sweden. Scand. J. For. Res.: 1–13. Taylor & Francis. doi:10.1080/02827581.2015.1005127.

Dutcă, I., Negrutiu, F., Ioras, F., Maher, K., Blujdea, V.N.B., and Ciuvat, L.A. 2014. The Influence of Age, Location and Soil Conditions on the Allometry of Young Norway Spruce (Picea abies L. Karst.) Trees. Not. Bot. Horti Agrobot. Cluj-Napoca **42**(2): 579–582. doi:10.15835/NBHA4229714.

Enquist, B.J., Brown, J.H., and West, G.B. 1998. Allometric scaling of plant energetics and population density. Nature **395**(6698): 163–165. Nature Publishing Group. doi:10.1038/25977.

Enquist, B.J., West, G.B., Charnov, E.L., and Brown, J.H. 1999. Allometric scaling of production and life-history variation in vascular plants. Nature **401**(6756): 907–911. Nature Publishing Group. doi:10.1038/44819.

Feldpausch, T.R., Lloyd, J., Lewis, S.L., Brienen, R.J.W., Gloor, M., Monteagudo Mendoza, A., Lopez-Gonzalez, G., Banin, L., Abu Salim, K., Affum-Baffoe, K., Alexiades, M., Almeida, S., Amaral, I., Andrade, A., Aragão, L.E.O.C., Araujo Murakami, A., Arets, E.J.M.M., Arroyo, L., Aymard C., G.A., Baker, T.R., Bánki, O.S., Berry, N.J., Cardozo, N., Chave, J., Comiskey, J.A., Alvarez, E., de Oliveira, A., Di Fiore, A., Djagbletey, G., Domingues, T.F., Erwin, T.L., Fearnside, P.M., França, M.B., Freitas, M.A., Higuchi, N., E. Honorio C., Iida, Y., Jiménez, E., Kassim, A.R., Killeen, T.J., Laurance, W.F., Lovett, J.C., Malhi, Y., Marimon, B.S., Marimon-Junior, B.H., Lenza, E., Marshall, A.R., Mendoza, C., Metcalfe, D.J., Mitchard, E.T.A., Neill, D.A., Nelson, B.W., Nilus, R., Nogueira, E.M., Parada, A., Peh, K.S.-H., Pena Cruz, A., Peñuela, M.C., Pitman, N.C.A., Prieto, A., Quesada, C.A., Ramírez, F., Ramírez-Angulo, H., Reitsma, J.M., Rudas, A., Saiz, G., Salomão, R.P., Schwarz, M., Silva, N., Silva-Espejo, J.E., Silveira, M., Sonké, B., Stropp, J., Taedoumg, H.E., Tan, S., ter Steege, H., Terborgh, J., Torello-Raventos, M., van der Heijden, G.M.F., Vásquez, R.,

Vilanova, E., Vos, V.A., White, L., Willcock, S., Woell, H., and Phillips, O.L. 2012. Tree height integrated into pantropical forest biomass estimates. Biogeosciences **9**(8): 3381–3403. Copernicus GmbH. doi:10.5194/bg-9-3381-2012.

Forrester, D.I., Bauhus, J., Cowie, A.L., and Vanclay, J.K. 2006. Mixed-species plantations of Eucalyptus with nitrogen-fixing trees: A review. For. Ecol. Manage. **233**(2–3): 211–230. doi:10.1016/j.foreco.2006.05.012.

Forrester, D.I., Benneter, A., Bouriaud, O., and Bauhus, J. 2017a. Diversity and competition influence tree allometric relationships - developing functions for mixed-species forests. J. Ecol. **105**(3): 761–774. doi:10.1111/1365-2745.12704.

Forrester, D.I., Tachauer, I.H.H., Annighoefer, P., Barbeito, I., Pretzsch, H., Ruiz-Peinado, R., Stark, H., Vacchiano, G., Zlatanov, T., Chakraborty, T., Saha, S., and Sileshi, G.W. 2017b. Generalized biomass and leaf area allometric equations for European tree species incorporating stand structure, tree age and climate. For. Ecol. Manage. **396**(396): 160–175. doi:10.1016/j.foreco.2017.04.011.

Fox, J., and Weisberg, S. 2011. An R companion to applied regression. Available from https://us.sagepub.com/en-us/nam/an-r-companion-to-applied-regression/book233899 [accessed 6 March 2017].

Harrington, R.A., and Fownes, J.H. 1993. Allometry and growth of planted versus coppice stands of four fast-growing tropical tree species. For. Ecol. Manage. **56**(1–4): 315–327. doi:10.1016/0378-1127(93)90120-C.

Hegyi, F. 1974. A simulation model for managing jack pine stands. *In* Growth Models for Tree and Stand Simulations. *Edited by* J. Fried. Royal College of Forestry, Stockholm. pp. 74–90.

Jucker, T., Bouriaud, O., and Coomes, D.A. 2015. Crown plasticity enables trees to

optimize canopy packing in mixed-species forests. Funct. Ecol. **29**(8): 1078–1086. doi:10.1111/1365-2435.12428.

Jucker, T., Caspersen, J., Chave, J., Antin, C., Barbier, N., Bongers, F., Dalponte, M., van Ewijk, K.Y., Forrester, D.I., Haeni, M., Higgins, S.I., Holdaway, R.J., Iida, Y., Lorimer, C., Marshall, P.L., Momo, S., Moncrieff, G.R., Ploton, P., Poorter, L., Rahman, K.A., Schlund, M., Sonké, B., Sterck, F.J., Trugman, A.T., Usoltsev, V.A., Vanderwel, M.C., Waldner, P., Wedeux, B.M.M., Wirth, C., Wöll, H., Woods, M., Xiang, W., Zimmermann, N.E., and Coomes, D.A. 2017. Allometric equations for integrating remote sensing imagery into forest monitoring programmes. Glob. Chang. Biol. **23**(1): 177–190. doi:10.1111/gcb.13388.

Kauppi, A., Kiviniitty, M., and Ferm, A. 1988. Growth habits and crown architecture of *Betula pubescens* Ehrh. of seed and sprout origin. Can. J. For. Res. **18**(12): 1603–1613. NRC Research Press Ottawa, Canada . doi:10.1139/x88-244.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., and Rubel, F. 2006. World Map of the Köppen-Geiger climate classification updated. Meteorol. Zeitschrift **15**(3): 259–263. doi:10.1127/0941-2948/2006/0130.

Lines, E.R., Zavala, M.A., Purves, D.W., and Coomes, D.A. 2012. Predictable changes in aboveground allometry of trees along gradients of temperature, aridity and competition. Glob. Ecol. Biogeogr. **21**(10): 1017–1028. Blackwell Publishing Ltd. doi:10.1111/j.1466-8238.2011.00746.x.

Loewe, V., González, M., and Balzarini, M. 2013. Wild cherry tree (Prunus avium L.) growth in pure and mixed plantations in South America. For. Ecol. Manage. **306**: 31–41. doi:10.1016/j.foreco.2013.06.015.

Parresol, B.R. 1999. Assessing Tree and Stand Biomass: A Review with Examples

and Critical Comparisons. For. Sci. **45**(4): 573–593. Available from http://www.sidalc.net/repdoc/A11144i/A11144i.pdf [accessed 11 November 2016].

Picard, N., Saint-André, L., and Henry, M. 2012. Manual for building tree volume and biomass allometric equations: from field measurement to prediction. FAO and CIRAD, Rome, Italy, and Montpellier, France. Available from http://www.fao.org/sustainable-forest-management/toolbox/tools/tool-detail/en/c/218049/ [accessed 16 August 2016].

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. Available from https://cran.r-project.org/web/packages/nlme/index.html [accessed 9 January 2017].

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., and Mommer, L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. **193**(1): 30–50. Blackwell Publishing Ltd. doi:10.1111/j.1469-8137.2011.03952.x.

Pretzsch, H. 2006. Species-specific allometric scaling under self-thinning: evidence from long-term plots in forest stands. Oecologia **146**(4): 572–583. Springer-Verlag. doi:10.1007/s00442-005-0126-0.

Pretzsch, H. 2014. Canopy space filling and tree crown morphology in mixed-species stands compared with monocultures. For. Ecol. Manage. **327**: 251–264. doi:10.1016/j.foreco.2014.04.027.

Pretzsch, H., and Biber, P. 2016. Tree species mixing can increase maximum stand density [1]. Can. J. For. Res. **46**(10): 1179–1193. NRC Research Press. doi:10.1139/cjfr-2015-0413.

Pretzsch, H., Block, J., Dieler, J., Dong, P.H., Kohnle, U., Nagel, J., Spellmann, H.,

and Zingg, A. 2010. Comparison between the productivity of pure and mixed stands of Norway spruce and European beech along an ecological gradient. Ann. For. Sci. **67**(7): 712–712. EDP Sciences. doi:10.1051/forest/2010037.

Pretzsch, H., and Schütze, G. 2009. Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: evidence on stand level and explanation on individual tree level. Eur. J. For. Res. **128**(2): 183–204. Springer-Verlag. doi:10.1007/s10342-008-0215-9.

Pretzsch, H., and Schütze, G. 2014. Size-structure dynamics of mixed versus pure forest stands. For. Syst. **23**(3): 560. doi:10.5424/fs/2014233-06112.

R Development Core Team. 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.r-project.org [accessed 14 September 2016].

Rozenberg, P., Franc, A., Bastien, C., and Cahalan, C. 2001. Improving models of wood density by including genetic effects: A case study in Douglas-fir. Ann. For. Sci. **58**(4): 385–394. EDP Sciences. doi:10.1051/forest:2001132.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Available from http://www.rstudio.com/ [accessed 14 September 2016].

Saha, S., Kuehne, C., and Bauhus, J. 2014. Intra- and interspecific competition differently influence growth and stem quality of young oaks (Quercus robur L. and Quercus petraea (Mattuschka) Liebl.). Ann. For. Sci. **71**(3): 381–393. Springer Paris. doi:10.1007/s13595-013-0345-1.

Sprugel, D.G. 1983. Correcting for Bias in Log-Transformed Allometric Equations. Ecology **64**(1): 209–210. Ecological Society of America. doi:10.2307/1937343.

Tabachnick, B.G., and Fidell, L.S. 2013. Using multivariate statistics. *In* 6th edition. Pearson Education, Boston.

Wang, J., Zhang, C., Xia, F., Zhao, X., Wu, L., von Gadow Wang, K., and Wang, A. 2011. Biomass Structure and Allometry of Abies nephrolepis (Maxim) in Northeast China. Silva Fenn. **45**(2): 211–226. Available from http://www.metla.fi/silvafennica/full/sf45/sf452211.pdf [accessed 22 July 2017].

Wirth, C., Schumacher, J., and Schulze, E.-D. 2004. Generic biomass functions for Norway spruce in Central Europe--a meta-analysis approach toward prediction and uncertainty estimation. Tree Physiol. **24**(2): 121–139. Oxford University Press. doi:10.1093/treephys/24.2.121.

Table 1. The characteristics of the investigated forest stands

Characteristics	Stand type	
	Mixed	**Pure**
Area (ha)	16.3	18.5
Latitude	45.525	45.534
Longitude	25.635	25.616
Altitude (m)	1050-1200	1180-1250
Soil type / Substratum	Dystric Cambisol / Sedimentary	Dystric Cambisol / Sedimentary
Slope (%)	28	27
Species composition	70% European beech 20% Norway spruce 10% Silver fir	90% Norway spruce 10% European beech
Age of the oldest Norway spruce trees (years)	18	18
No. of stems (per hectare)*	3950[a]	3700
Mean DBH (SD), in cm	7.8 (2.9)	8.6 (2.1)
Mean height (SD), in m	8.3 (3.1)	8.4 (2.2)
Standing volume (in m^3)	141[b]	136
No. of sampled trees	25	25

* Includes all trees within the plot.

[a] Stem densities (stocking) by species were: European beech, 3442/ha; Norway spruce, 508/ha.

[b] Standing volumes over bark by species were: European beech, 118 m^3; Norway spruce, 23 m^3.

Table 2. ANCOVA main effects and covariate interactions (*p* values) for pure vs. mixed species composition

DV	Covariates	SC effect (1)	SC effect (2)	Interactions			
				T_d:CI:SC	CI:SC	T_d:SC	T_d:CI
ln(AB)	$\ln(DBH)^2$, CI	0.005	0.005	0.274	0.103	0.356	0.882
	ln(H), CI	0.042	0.045	0.446	0.692	0.751	0.569
	$\ln(DBH^2H)^2$, CI	0.002	0.002	0.375	0.211	0.330	0.939
ln(ST)	$\ln(DBH)^2$, CI	0.663	0.698	0.322	0.005	0.202	0.874
	ln(H), CI	0.132	0.139	0.298	0.983	0.799	0.595
	$\ln(DBH^2H)^2$, CI	0.099	0.076	0.221	0.001	0.012	0.870
ln(BR)	$\ln(DBH)^2$, CI	0.0009	0.001	0.806	0.980	0.848	0.868
	ln(H), CI	0.017	0.024	0.814	0.383	0.260	0.634
	$\ln(DBH^2H)^2$, CI	0.001	0.002	0.885	0.940	0.747	0.839
ln(ND)	$\ln(DBH)^2$, CI	0.027	0.028	0.953	0.512	0.465	0.818
	ln(H), CI	0.119	0.107	0.816	0.306	0.167	0.612
	$\ln(DBH^2H)^2$, CI	0.032	0.033	0.961	0.514	0.401	0.798

Abbreviations: DV – dependent variable; CI – competition index; SC – species composition; T_d – dendrometric covariate (tree dimensions) that could be $\ln(DBH)^2$, ln(H) or $\ln(DBH^2H)^2$; SC effect (1) – main SC effect without interactions (Eq. 3); SC effect (2) – main SC effect with interactions (Eq. 5).

Table 3. The prediction bias resulted from the main SC effect (see Eq. 6).

Model	**Bias (%)**
AB=*f*(DBH)	-15.47**
AB=*f*(H)	-19.08*
AB=*f*(DBH, H)	-19.28***
BR=*f*(DBH)	-28.11***
BR=*f*(H)	-28.29**
BR=*f*(DBH, H)	-29.91***
ND=*f*(DBH)	-19.93*
ND=*f*(DBH, H)	-21.15*

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ here are presented for those main effects that were sources of bias (see Fig. 1). Note: The prediction bias resulted from the main effect was calculated only for models showing significant ANCOVA and non-significant interactions.

List of figures:

Fig. 1.

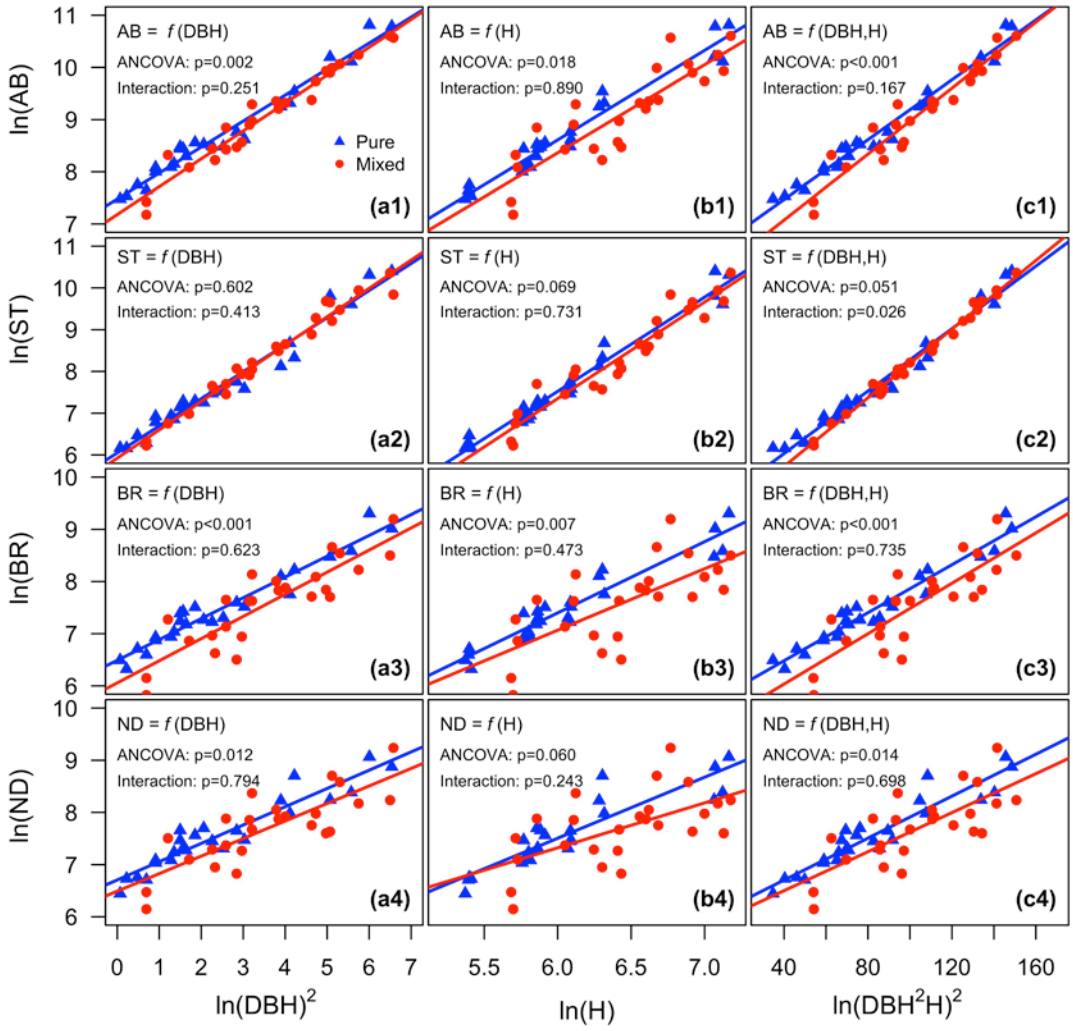

AB = f (DBH)
ANCOVA: p=0.002
Interaction: p=0.251
Pure
Mixed
(a1)
AB = f (H)
ANCOVA: p=0.018
Interaction: p=0.890
(b1)
AB = f (DBH,H)
ANCOVA: p<0.001
Interaction: p=0.167
(c1)
ST = f (DBH)
ANCOVA: p=0.602
Interaction: p=0.413
(a2)
ST = f (H)
ANCOVA: p=0.069
Interaction: p=0.731
(b2)
ST = f (DBH,H)
ANCOVA: p=0.051
Interaction: p=0.026
(c2)
BR = f (DBH)
ANCOVA: p<0.001
Interaction: p=0.623
(a3)
BR = f (H)
ANCOVA: p=0.007
Interaction: p=0.473
(b3)
BR = f (DBH,H)
ANCOVA: p<0.001
Interaction: p=0.735
(c3)
ND = f (DBH)
ANCOVA: p=0.012
Interaction: p=0.794
(a4)
ND = f (H)
ANCOVA: p=0.060
Interaction: p=0.243
(b4)
ND = f (DBH,H)
ANCOVA: p=0.014
Interaction: p=0.698
(c4)
ln(AB)
ln(ST)
ln(BR)
ln(ND)
ln(DBH)2
ln(H)
ln(DBH2H)2

Fig. 2.

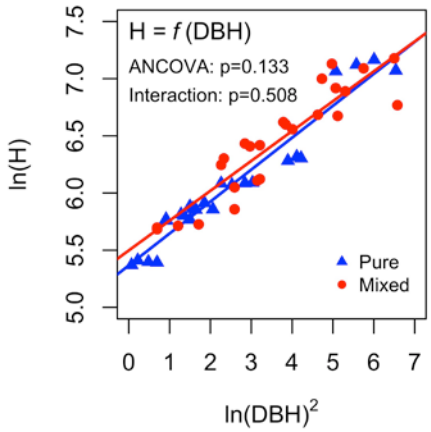
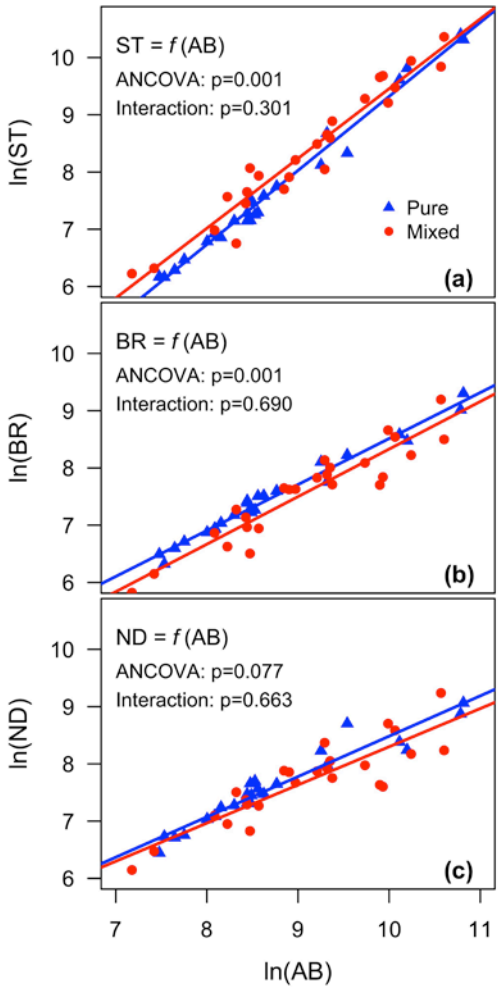

H = f (DBH)
ANCOVA: p=0.133
Interaction: p=0.508
Pure
Mixed
ln(H)
ln(DBH)2
5.0
5.5
6.0
6.5
7.0
7.5
0
1
2
3
4
5
6
7

Fig. 3.


ST = f (AB)
ANCOVA: p=0.001
Interaction: p=0.301
Pure
Mixed
(a)
BR = f (AB)
ANCOVA: p=0.001
Interaction: p=0.690
(b)
ND = f (AB)
ANCOVA: p=0.077
Interaction: p=0.663
(c)
ln(ST)
ln(BR)
ln(ND)
ln(AB)
6
7
8
9
10
11